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AN EVALUATION OF SEMEN PRODUCTION IN BRAHMAN BULLS.

Louisiana State University and Agricultural and Mechanical College, Ph.D., 1969
Agriculture, animal culture

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AN EVALUATION OF SEMEN PRODUCTION IN BRAHMAN BULLS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Animal Science

by
Robert Chapman Kirst
B.S., Louisiana State University, 1960
M.S., University of Florida, 1964
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ABSTRACT

This three-phase study was designed to investigate the effects of ambient temperature, short-term heat stress, and an exhaustion technique on semen production and semen quality of Brahman bulls.

Analysis of the data used to study the effect of ambient temperature on semen quality disclosed highly significant differences in semen volume in collections made from fourteen 4-year-old Brahman bulls.

The effects of bulls and months on semen concentration and spermatozoa per ejaculate were found to be highly significant.

Differences in the percentage of motile sperm due to weeks, months, and bull were found to be highly significant.

Highly significant differences were noted in the percentage of abnormal spermatozoa for months, weeks within months, and bull.

The effect of month and bull on the percent of dead sperm was significant.

Body temperature was significantly affected by months, weeks within months, and bull.

No differences in semen quality were found between breeds when four Brahman bulls and four Angus bulls were exposed to 40°C. at 35% humidity for 12 hours. No weekly differences were found in volume of ejaculate or percent of motile sperm. It appears that the increase in percentage of abnormal and dead sperm for the bulls exposed to climatic stress was due to short exposure to high temperatures in the climate-control chamber.

Results seem to indicate that the quality of Brahman semen decreases under high ejaculation frequencies. With the electro-ejaculation technique used the spermatozoa reserves appeared virtually inexhaustible. However, the semen production and semen quality in Brahman bulls are adversely affected by heat stress.

INTRODUCTION

In recent years, the need to increase the efficiency of reproduction in beef cattle has prompted much research on the reproductive phenomena of the female. However, only limited work has been done regarding semen production in the male.

An increased knowledge of those factors, both genetic and environmental, which affect semen production and quality would allow for more efficient use of our bull power. Such knowledge would make possible more accurate selection of bulls with maximum reproductive potential. Greater efficiency in use of sires could be obtained both in natural mating systems and artificial insemination with information on factors influencing spermatogenesis and their effect on semen quality.

Research with bulls of the dairy breeds has shown that numerous factors--such as light, temperature, nutrition, and frequency of collection--have marked effects on semen production and quality. However, caution should be used in evaluating beef sires based on information obtained from dairy sires.

The objectives of this study were: (a) to

determine the effects of season on semen production and semen quality of Brahman bulls; (b) to evaluate the influence of short-term heat stress on semen production of Angus and Brahman bulls; (c) to study semen quality and numbers of spermatozoa in the reproductive tracts of Brahman bulls through use of a partial-exhaustion technique.

REVIEW OF LITERATURE

Numerous investigations into semen quality and factors affecting it have been made. Donham et al. (1931) and Herman and Sevanson (1941) found that marked variations in seminal specimens from the same bull made it impossible to determine from a single examination what might be expected from future specimens taken from the same bull. Shukla and Bhattacharya (1949) in studies on the semen characteristics of the Indian breeds of livestock found considerable variation in semen quality between breeds.

Effects of Electro-ejaculation

Only limited data are available on the use of electro-ejaculation equipment for collecting semen from bulls or its effects on semen quality as compared with the artificial vagina.

Watson (1964) used electro-ejaculation procedures to collect semen from 110 Hereford and Devon range bulls. He found a mean volume of 15.8 ml. of semen per ejaculate and a mean of 5.22×10^9 spermatozoa per ejaculate. Parish and Murphree (1956), used an electro-ejaculation technique to collect 1,131 samples of bull semen. They found that most of the samples collected by electro-ejaculation were

of a larger volume but contained relatively fewer sperm per ml. when compared with semen samples collected using the artificial vagina. Very little difference was found between the two collection methods in the total sperm count per ejaculate. Hafs and Knisley (1960) compared electro-ejaculation to collection with an artificial vagina on five dairy and two beef bulls. They found that electro-ejaculation resulted in a larger volume of semen but lower sperm concentration. Total motile sperm per ejaculate using the electro-ejaculator was essentially equivalent to ejaculates using the artificial vagina, 10.1 billion and 9.9 billion, respectively. Differences among breeds, among seasons, and among season by breed interactions were insignificant for volume, percent motility, sperm per ml., sperm per ejaculate, and motile sperm per ejaculate. The interactions between seasons and bulls were significant for all criteria except percent motility indicating that sensitivity to seasonal changes differs among bulls.

The effect of method of semen collection and tranquilization on semen quality was studied by Wells et al. (1966). Three groups, each with four Holstein bulls, were used to compare the artificial vagina, electro-ejaculation, and electro-ejaculation plus tranquilizer. Samples collected using the artificial vagina were higher in live

sperm per ejaculate than either of the other two methods. No significant differences were found in initial motility rate, percent motility, or percent abnormal sperm. The results indicated that tranquilizers increased the quality of semen obtained by electro-ejaculation.

Seasonal Effects

The effect of season on semen quality has been the subject of numerous investigations. Observations over a period of one year on the semen characteristics of Khillar cattle by Kodagali (1962, 1963) revealed semen quality was superior in cold weather, followed by the wet season and the hot season, respectively.

Swanson and Herman (1944) found monthly variations in volume, concentration, and percent abnormal spermatozoa were non-significant for dairy bulls. Initial motility was lower in winter than in the spring and summer; however, this was believed due to the effects of adverse winter weather upon the aged bulls.

Holstein, Jersey, Guernsey, and Ayrshire bulls were used by Erb et al. (1942) to study the seasonal variation in semen quality of dairy bulls. Differences between bulls and between months was highly significant for semen volume, initial motility, and concentration. Semen quality was significantly superior during spring and significantly inferior during summer. Semen produced during the fall was

not significantly different from semen produced in winter.

Mercier and Salisbury (1946) found highly significant breed differences between Holstein and Guernsey bulls in the proportion of abnormal sperm. Significant monthly differences were found in volume, percent motility, concentration, and percent abnormals but not in motility rate or total sperm per ejaculate.

Semen from three beef Shorthorn and three milking Shorthorn bulls was studied by Phillips et al. (1945) at two-week intervals throughout a year. They found the variation due to season was not significant in motility, volume, total abnormal sperm, and survival in storage.

Seasonal variations in the characteristics of Hariana bull spermatozoa were studied by Mukherjee and Singh (1966). Four semen samples were obtained over 13 days from each of four bulls at each of four seasons (December, March, May, and July). Between seasons only the spermatozoa head shape and length varied significantly. Head length decreased and head width increased with an increase in environmental temperature. The percentage of dead sperm did not vary significantly between bulls or season.

Lasley (1943) and Lasley and Bogart (1943) found that Hereford bulls gave higher quality semen as the breeding season progressed from May 1 to September 15. Volume, percent live spermatozoa, and percent resistant sperm

increased during this period but concentration and percent abnormal did not.

Milicevic (1965) investigated the seasonal variation in the quality of bull semen using data collected from 18 Simmental bulls. He found that high environmental temperatures reduced ejaculation volume. Low temperatures adversely affected pH, concentration, motility, and viability.

Seasonal variation in the reproductive capacity of bulls was studied by Anderson (1945) for 27 months. Highly significant monthly differences were noted for density and motility. Considerable variation was also noted between bulls and farms. It was postulated that warm conditions stimulate semen production.

Examination by Donaldson (1963) of Santa Gertrudis, Shorthorn, Shorthorn X Devon, and Brahman crossbred bulls revealed that bulls with low scores for testicular tone had poorer quality semen than those with higher scores. Significantly higher ratings were noted for testicular tone, sperm density, motility, and semen color in July than in March. No differences in percent live spermatozoa and wave motion were found. It was felt that these findings were due to heat stress. It was concluded that differences could exist between British breeds and animals with a proportion of Bos indicus blood.

Several studies have based judgments of semen quality on the percent non-returns. Considerable seasonal variation was found by Erb et al. (1940) in a study of the breeding efficiency in the Purdue University dairy herd over a 20-year period. May was found to have the highest efficiency and August the lowest. In a later study of the seasonal changes in fertility of dairy bulls in northwest Washington, Erb and Waldo (1952) found highly significant monthly variation. January had the lowest average non-return rate and September the highest. Johnston and Branton (1953) found similar effects indicating a carry over effect of hot weather on semen quality.

Heat Stress

Artificially imposed heat stress has been used to determine the effect of temperature on semen production and quality. The effect of scrotal insulation on the semen of 12 Hereford bulls was studied by Austin et al. (1961). Insulation of the scrotum raised the mean scrotal skin temperature approximately 3°F. In both treated groups (24 and 72 hours), the number of live sperm decreased to approximately 65 percent of the controls in the second and third week after insulation. The percent normal sperm dropped to approximately 60 percent of the controls in the same period. Treated animals returned to control levels for those factors by the sixth week. In the 72-hour treatment

group, sperm motility and concentration decreased during the fourth to seventh week.

Langerlof (1934) reported increased numbers of abnormal spermatozoa 10-40 days after four to nine days of scrotal insulation in three bulls. Increased abnormal sperm followed by temporary sterility of four months' duration in bulls insulated for 11 and 16 days was also reported.

Casady et al. (1953) studied the effect of exposure to high ambient temperature on spermatogenesis of four young Guernsey bulls. The bulls were continuously exposed to various ambient temperatures in two experimental chambers. Temperatures ranged from 70 to 99°F. in one and 70 to 52°F. and 52 to 86°F. in the other. Average initial motility, sperm concentration, and total sperm numbers decreased markedly for all animals either during or following exposure to high ambient temperature. Exposure to approximately 100°F. resulted in impaired spermatogenesis in two bulls. Two bulls exposed to 86°F. for five weeks showed impaired spermatogenesis, producing ejaculates practically void of sperm for as long as two months following exposure.

Jersey bulls were subjected to 35-36°C. for eight hours daily for 26 weeks by DeAlba and Riera (1966). Results indicated that the hot temperature exposure retarded fertility. Reduced semen quality was indicated by reduced

numbers of normal and live spermatozoa. They associated the poor semen quality with impaired spermatogenesis.

Studies were conducted by Johnston et al. (1963) to determine the effect of short-period exposure to cyclic hot climatic conditions on semen characteristics of Holstein, Brown Swiss, and respective Red Sindhi crossbred bulls. Bulls were exposed for eight hours daily to a maximum of 104°F. and 30mm Hg. and a minimum of 82°F. and 22mm Hg. High temperature and humidity had detrimental effects on semen quality as evidenced by low initial motility, concentration, and total numbers of spermatozoa and increased sperm abnormalities. Decreased semen quality was more marked in the purebreds. Recovery from the deleterious effects was more rapid in the Red Sindhi.

Skinner and Louw (1966) carried out a series of experiments to determine the critical duration of high ambient temperature (40°C.) required to affect spermatogenesis adversely in the bovine. A comparison of Bos indicus and Bos taurus breeds was made as well as a study of the site and nature of the damage to the spermatogenic cycle. Optimum spermatogenesis was impaired in both species but most severely in the Bos taurus. An exposure period of as little as 12 hours was found to be critical. A significant decrease in motility and percentage of live spermatozoa was noted together with an increase in abnormal spermatozoa.

Exhaustion

One important difficulty in conducting exhaustion studies is determining when exhaustion has occurred. The use of protoplasmic droplets as an indication that immature spermatozoa are being collected is of doubtful value. Langerlof (1936) stated that protoplasmic droplets found in semen samples indicated immature sperm; however, Kirillov and Morozov (1933) found no evidence that protoplasmic droplets serve as an indication of age of sperm or degree of exhaustion.

Using phosphorus-32 as a marker, Koefoed-Johnson L9160) found that frequency of collection had no effect on the time required for sperm formation and epididymal passage in the bull. First appearance of labelled DNA occurred from 48-51 days after injection and reach a peak between 52-69 days after injection.

In order to determine the number of spermatozoa in various parts of the reproductive tract of bulls, several investigators have made direct counts using excised tracts. Through examination of excised tracts Bialy and Smith (1956) and Bialy and Smith (1958) found an average of 821,955 x 10^3 sperm in 18 combined ampulae and vasa deferentia. The average number of sperm in the cauda epididymi obtained from 25 specimens was 8,880,632 x 10^3.

Macillan and Hafs (1968) reported an average gonadal

sperm concentration of 55.1 million per gram of testicular parenchyma in 1-year-old Holstein bulls. Almquist and Amann (1961), using mature Holstein bulls, found that gonadal sperm concentration was 54.8 million per gm. and gonadal sperm numbers were 40.4 billion per bull. It was estimated by Willet and Ohms (1957) that aged bulls produced an average of 467 million sperm per gram of testicular tissue per week.

Investigations concerning total numbers of sperm per ejaculate as well as total numbers per exhaustion have produced conflicting results. Hafs et al. (1962) obtained a total of 19.6 billion sperm in the first ejaculate from a mature bull following intensive sexual preparation. Bratton et al. (1959) reported an average of 1.3 billion sperm per ejaculate from young Holstein bulls collected once every two weeks. Singh and Prabhu (1963a) collected two ejaculates either once or twice weekly from Kumauni bulls. They reported the average sperm per ejaculate was 747 million for bulls collected twice weekly. An average of 1.947 billion sperm per ejaculate was reported by Boyd and Van Demark (1957) for two-year-old Holstein bulls.

Boyd and Van Demark (1957) found an average of 18.1 billion sperm per exhaustion from two-year-old Holstein bulls. A partial exhaustion test by Walton and Edwards (1938) produced an average of 15.627 billion sperm per bull.

Exhaustion trials reported by Hale et al. (1953) used an average of 41 semen collections per trial (range 22-77). They obtained an average of 47.8 billion sperm per bull per trial. Within one week semen quality and quantity was reportedly restored to pre-exhaustion levels.

The influence of frequency of collection on semen quality has been the subject of a number of investigations. In two studies by Singh and Prabhu (1963a, b) using Kumauni bulls an adverse effect on semen quality was noted as frequency of collection increased from one to four times per week. Decreased semen volume, initial motility, and sperm concentration was observed as collection frequency increased.

Adult Hariana bulls were used by Singh and Prabhu (1963c) to compare the effects of collecting two ejaculates once a week, two ejaculates per day twice a week, and one ejaculate per day four times a week. A decrease was found between all frequencies in volume, concentration and total sperm. A significant decrease in concentration and total sperm per ejaculate was noted between two ejaculates per day twice a week and one ejaculate per day four times per week.

Collection frequencies of once, twice, and three times per week were compared by Baker et al. (1955). No significant differences attributable to frequency of

collection were found for semen volume, concentration, percent motile sperm, semen pH, percent abnormal sperm, or total sperm per ejaculate.

Investigations by Almquist and Cunningham (1967) indicated that as ejaculation frequency increased from one to six times weekly, semen volume per ejaculate decreased and weekly total sperm and total motile sperm output increased significantly. Sperm concentration and initial motility were not significantly affected.

Almquist et al. (1963) found no significant effect due to ejaculation frequency upon the freezability of sperm from Holstein bulls collected one time or six times weekly. Similar results were reported by Cunningham et al. (1967) for young Hereford and Angus bulls.

SEMEN COLLECTION TECHNIQUE

Personal communication with both veterinarians and personnel at the Louisiana Animal Breeders' Cooperative, Inc., indicated that semen collection with an electro-ejaculator was difficult with Brahman bulls. Thus, a technique for collecting semen from Brahman bulls was developed and refined for use in this study.

Materials used in semen collections were:

1. Squeeze chute
2. Nicholson Trans-Jector
3. Volumetric centrifuge tube (15cc)
4. Funnel (short stem)

The criteria for the selection of a squeeze chute were:

1. sufficient strength to provide maximum protection to the collector and maximum restraint of the bull
2. provide ample leg room so that the bull could distribute his weight evenly
3. strong bottom to provide secure footing
4. a drop panel for access to the genitals.

The bull was brought into the chute and squeezed to facilitate collection and to prevent the bull from injuring himself. The bull's head was not restrained as it was

found that when "given his head" a bull was less likely to lie down. An iron pipe was placed horizontally behind the bull at about the level of the hocks to provide something against which the bull could push. The pubic hair was trimmed from the sheath with scissors and the area cleaned with clear water. The 2.5-inch-diameter probe was lubricated lightly with Ivory soap and inserted into the rectum. The Trans-Jector was turned on and the output slowly increased to 1.0 volt. The 1.0-volt output level was maintained for approximately 10 seconds then slowly returned to zero. The voltage was again slowly increased from zero to 1.0 volt and then slowly returned to zero. This increase to 1.0 volt and return to zero was repeated approximately three times. The stimulus was then increased by approximately 0.5-volt steps. Each 0.5-volt increase in stimulus was repeated approximately three times before proceeding to the next voltage increase. This procedure was repeated until ejaculation occurred, usually at an output level of less than 5 volts. If the 5-volt output level had been reached without ejaculation, the Trans-Jector was turned off; and the bull was given approximately 5 minutes rest, after which the entire procedure was repeated. If ejaculation had not occurred on the second trial by a 5-volt level, the voltage was increased by 0.5-volt steps until ejaculation did occur. The only difficulty encountered

with this technique was the failure of the Brahman bulls to achieve an erection.

The successful application of this technique to Brahman bulls was attributed to a slow, gradual increase in electrical stimulation. The frequent failure of many technicians to secure an ejaculate from Brahman bulls with the electro-ejaculator is apparently due--at least in part--to a general tendency to increase the voltage of electrical stimulation too quickly to above optimum levels.

This same technique was tried on bulls of the other major beef breeds with total success, including erection.

SEMEN EVALUATION PROCEDURES

All semen samples collected in the three phases of this study were handled and evaluated using the procedures given below. All samples were collected in 15 ml. volumetric test tubes wrapped with paper toweling to prevent damage to the spermatozoa by light and cold shock.

Volume

The volume of the ejaculate was recorded immediately after collection.

Percent Motility

Immediately after collection, a light microscope equipped with a stage warmer set at 100°F. was used in assigning a motility score to the sample. A clear slide was placed on the stage warmer, and a drop of sodium citrate solution (3%) was placed in the center of the slide. The tip of a clear stirring rod was dipped into the fresh semen sample and the adhering semen quickly mixed with the sodium citrate drop on the slide. A cover slip was then placed over the mixture and the slide examined, first under low power (10X), then under high power (43X). A motility score of from 0 to 100 was then assigned to the sample.

Percent Dead

The eosine B-fast green stain used in making the slides for the live-dead evaluation was prepared by dissolving 2.0 gm. of fast green F.C.F. and 0.8 gm. of eosine (bluish, H_2O soluble) in 100 ml. of M/8 phosphate buffer (pH 7.3-7.4). The buffer was prepared by pipetting 20 ml. of M/8 monobasic sodium phosphate solution into a 100 ml. volumetric flask and adding M/8 dibasic sodium phosphate to the 100 ml. mark.

The glass slides (one end frosted) used in making the staining procedure were thoroughly cleaned in a solution of 100 ml. of alcohol and 2 ml. of hydrochloric acid.

After the motility evaluation was made, a drop of stain was placed in the center of a clean slide. A clean glass sitrring rod was dipped into the semen sample and the adhering semen mixed with the stain drop. A second clean slide was placed directly on the first slide. The two slides were quickly separated, using a sliding action, and placed on a hot plate (150°F.) for drying. The slides were then put in a slide box for transport back to the laboratory where the live-dead counts were made.

The prepared slides were examined under a light microscope at high power (43X). Those spermatozoa with darkly stained heads were considered dead while those sperm with heads devoid of stain were considered live. All sperm

in at least five fields were counted. The totals for both live and dead cells were kept on a hand counter, and the percent dead cells was calculated from those totals.

Concentration and Total Sperm per Ejaculate

The concentration of each semen sample was determined using an AO Spencer Bright-Line hemocytometer. A dilution rate of 1-200 was employed with a diluting fluid of normal saline and 1% chlorazine. The semen sample was drawn into the diluting pipette to the 0.5 mark. After removing the pipette from the semen sample, the fluid was drawn into the expanded bulb. The pipette was wiped to remove any cells adhering to it and was then dipped into the diluting fluid and the fluid drawn to the 1.01 mark. By holding the pipette between the thumb and index finger and shaking, the semen and diluting medium were mixed.

A clear cover glass was placed on the counting chamber and rubbed into close contact with the supporting ribs of the chamber. The first drop of the diluted semen was discarded and the second drop was placed on the metal surface at the edge of the cover glass. Care was taken to prevent overflow of the chamber. If overflow did occur the chamber was cleaned and refilled.

In order to allow the cells to settle to the bottom of the chamber, the preparation was set aside for approximately 1.0 minute. The chamber was examined with the 10X

objective to insure that the cells were evenly distributed and the counting was then done using the 43X objective.

Five large squares (80 small squares) diagonally across the field were counted. Only those cells were counted whose heads lay within the large square. Those sperm with their heads on the triple line bordering the large squares were counted only on the top and left triple lines. The formula used for determining the number of cells per cu. mm. was $N = C \times \frac{4000}{S} \times D$. (N = number of cells per cubic millimeter, C = number of cells counted, S = number of small squares counted, and D = dilution.) The number of spermatozoa per cubic millimeter times 1000 gave the number of cells per milliliter while the number of cells per milliliter times the volume of the semen sample collected equalled the number of spermatozoa per ejaculate.

Percent Abnormal

The percentage of abnormal spermatozoa was determined at the same time as the number of cells per cubic millimeter was found using the hemocytometer. The number of cells in 80 small squares was totalled on a counter in the right hand and the number of abnormals totalled on a counter in the left hand. The percent abnormal sperm was calculated from these figures.

PART I

EFFECTS OF AMBIENT TEMPERATURE ON SEMEN QUALITY AND SEMEN PRODUCTION IN BRAHMAN BULLS

Introduction

This experiment was undertaken to determine the influence of seasonal effects on the production and quality of semen from Brahman bulls. Additional knowledge in this area might allow for the planning of more workable mating programs. This experiment was conducted over an 8-month period from August 1967, through March 1968, at the L.S.U. R.O.P. barn, Dean Lee Drive, Baton Rouge, Louisiana.

Material and Methods

Fourteen Brahman bulls approximately 4 years of age were used in this study. Six of the bulls were from the L.S.U. herd, three bulls were provided by the G. L. Paret Ranch, Lake Charles, Louisiana; and five by the Bar-M Ranch, Covington, Louisiana.

The bulls were all maintained in a community pasture of approximately 5 acres which provided exercise room and limited grazing. Beginning as yearlings, all bulls were

fed twice daily a ration designed for proper growth and development. During periods when grazing was not available, grass hay was provided daily. All bulls had free access to water and minerals.

Weekly semen collections were made from all bulls during the 8-month experimental period. All semen samples were collected using the collection technique previously described. Semen was evaluated as outlined earlier. Rectal temperature was determined for each bull immediately prior to semen collection. All data were analyzed according to the methods described by Snedecor (1956).

Results and Discussion

Three orthogonal comparisons of treatment totals for month effect were made for all factors studied. These comparisons were: (1) August versus all other months; (2) September, October, and November (Fall) versus December, January, and February (Winter); and (3) Fall and Winter versus March.

Highly significant differences ($P < .01$) in semen volume were noted for months, weeks within months, and bulls, indicating large variations in semen volume due to these factors (Table I).

Orthogonal comparisons of grouping of months into seasons revealed that a significantly greater ($P < .01$)

TABLE I

ANALYSIS OF VARIANCE FOR THE EFFECT OF MONTH ON VOLUME OF SEMEN

Source	df	SS	MS
Total	391	941.2506	
Weeks	27	142.8463	5.2906**
Months	7	42.8615	6.1230**
Weeks/months	20	99.9848	4.9992**
Bull	13	145.6402	11.2030
Error	351	652.7641	1.8597

**P < .01

TABLE II

ANALYSIS OF VARIANCE FOR THE EFFECT OF MONTH ON TOTAL SPERM NUMBERS

Source	df	SS	MS
Total	321	8,774,877.1813	
Weeks	27	1,407,728.8227	52,138.1045**
Months	7	789,645.1104	112,806.4443**
Weeks/months	20	618,083.7123	30,904.1856
Bull	13	1,156,621.8177	88,970.9090**
Error	351	6,210,526.5409	17,693.8078

**P < .01

volume of semen was obtained in the fall than in winter. Volume was also significantly greater ($P < .01$) in March than in the fall and winter. These results would indicate that semen volume is reduced during periods of cold, wet weather. These findings are in agreement with the results of Erb et al. (1940) and Mercier and Salisbury (1946) in which monthly differences in semen volume were found when semen was collected with an artificial vagina. It should be noted, however, that these findings are not in agreement with the results of Swanson and Herman (1944) for semen collected with the artificial vagina or with the findings of Hafs and Knisley (1960) for semen collected by electro-ejaculation.

It is the opinion of this writer that a large part of the variation found in this study was probably due to the method of collection. Electro-ejaculation stimulated the release of large amounts of sperm-free seminal plasma. It was difficult to collect semen samples free of excess seminal fluids, particularly from those bulls which failed to have an erection.

The effects of months and bull on total spermatozoa per ejaculate were highly significant ($P < .01$) (Table II). Similar results were reported by Milicevic (1965) using Simmental bulls. Mercier and Salisbury (1946), however, found no monthly variation in sperm per ejaculate.

Orthogonal comparisons of treatment totals were made grouping months into the three previously described seasonal categories. The results of these comparisons showed that significantly greater ($P < .01$) numbers of sperm per ejaculate were produced in March than in the fall and winter months. The other comparisons were found to be non-significant.

Month and bull were found to have a highly significant effect ($P < .01$) on sperm concentration as shown in Table III. When monthly means were grouped to correspond to seasons, highly significant differences ($P < .01$) in concentration were found between August and all other months and between fall and winter and March. Sperm concentration was greater in the other months studied than in August and greater in March than in the fall and winter months. Monthly means for sperm concentration are plotted in Figure I. These figures show that the lowest mean concentration was in August with an increase to October, followed by a decline to a low in December. From December to March, an increase in mean concentration was noted, reaching a maximum for all months in March. These findings indicate that sperm concentration is influenced by high and low ambient temperatures. Studies by Mercier and Salisbury (1946) and Erb et al. (1940) indicated highly significant monthly differences in concentration. Milicevic (1965)

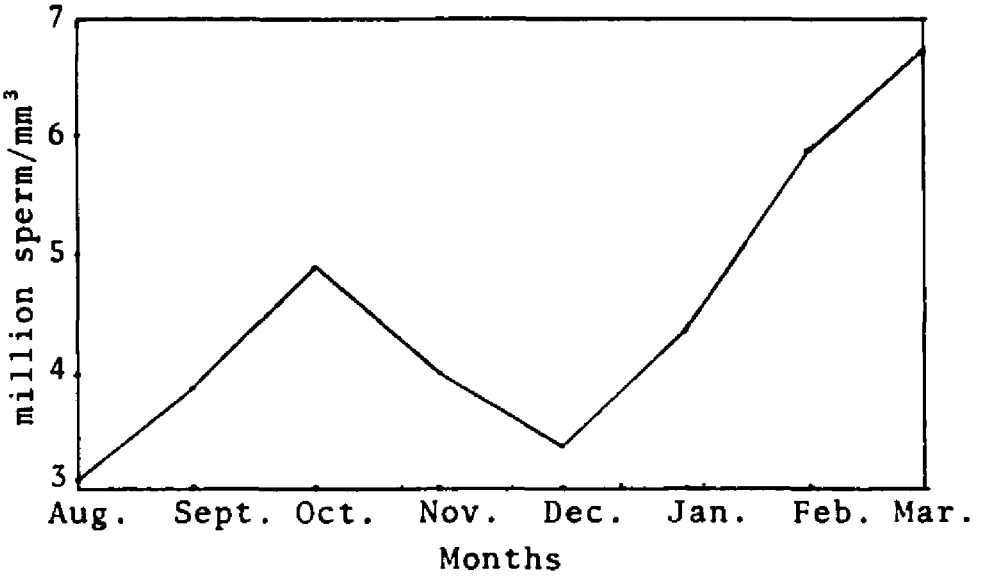

FIGURE I

Mean Concentration of Spermatozoa

TABLE III

ANALYSIS OF VARIANCE FOR THE EFFECT OF MONTH ON SPERM PER MM^3 OF SEMEN

Source	df	SS	MS
Total	391	437,444.3164	
Weeks	27	70,607.4592	2,615.0910**
Months	7	47,666.7899	6,809.5414**
Weeks/months	20	22,940.6693	1,147.0334
Bull	13	65,422.1735	5,032.4748**
Error	351	301,414.6837	858.7312

**P < .01

TABLE IV

ANALYSIS OF VARIANCE FOR THE EFFECT OF MONTH ON PERCENT MOTILITY

Source	df	SS	MS
Total	391	104,623.4057	
Weeks	27	15,971.6199	591.5414**
Month	7	12,045.7863	1,720.8266**
Weeks/months	20	3,925.8336	196.2916
Bull	13	15,301.9771	1,177.0751**
Error	351	73,349.8087	208.9738

**P < .01

reported that concentration was adversely affected by low temperatures.

Differences in the percentage of motile sperm due to months and bull were found to be highly significant ($P < .01$) (Table IV). The percent motility was significantly lower ($P < .01$) in August than in the other months studied. No significant differences were found for fall and winter versus March. These findings indicate that high temperature and humidity in August had an adverse effect on sperm motility. Figure II gives the monthly means for percent motility. Indications are that increases in sperm motility correspond with the decrease in temperature beginning in early fall. Results of research by Donaldson (1963) are in agreement with the results of this study. Milicevic (1965) reported that low temperatures adversely affected motility. Mercier and Salisbury (1946), Phillips et al. (1945), and Anderson (1945) found monthly and seasonal differences in sperm motility to be non-significant.

Highly significant ($P < .01$) differences for months, weeks within months, and bull were found in the percent of abnormal spermatozoa (Table V). Orthogonal comparisons for August versus all other months, fall versus winter and fall and winter versus March were significantly different ($P < .01$) from each other in the percent of

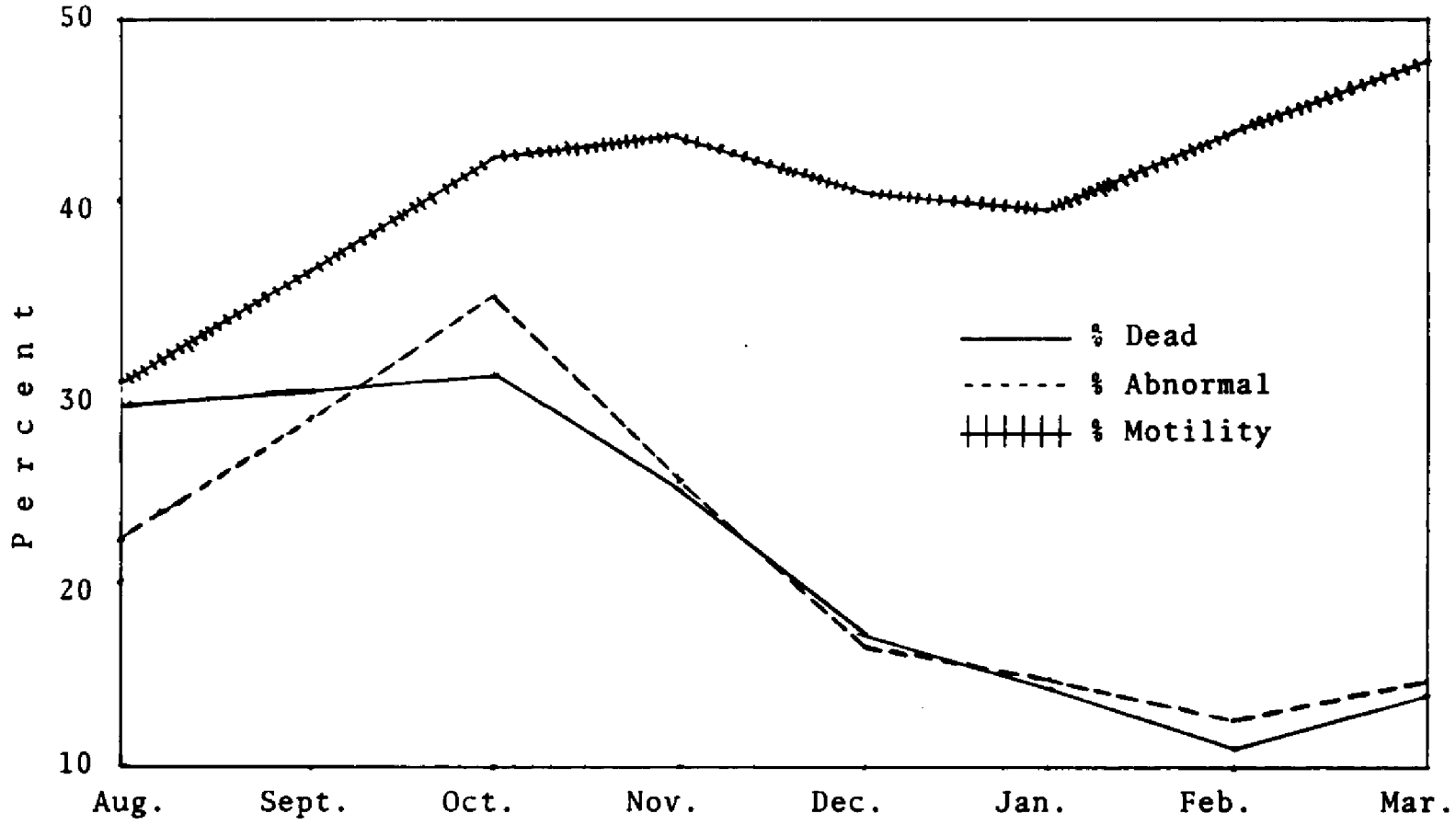

FIGURE II

Monthly Means for Percent Motility, Percent Abnormals and Percent Dead Sperm

TABLE V

ANALYSIS OF VARIANCE FOR THE EFFECT OF MONTH ON PERCENT OF ABNORMAL SPERM

Source	df	SS	MS
Total	391	60,285.3082	
Weeks	27	29,851.8924	1,105.6256**
Months	7	19,125.9746	2,732.2820**
Weeks/months	20	10,725.9178	536.2958**
Bull	13	4,257.8246	327.5249**
Error	351	26,175.5912	74.5743

**P < .01

TABLE VI

ANALYSIS OF VARIANCE FOR THE EFFECT OF MONTH ON PERCENT OF DEAD SPERM

Source	df	SS	MS
Total	391	74,066.5270	
Weeks	27	25,341.1112	938.5596**
Months	7	21,469.8971	3,067.1281**
Weeks/months	20	3,871.2141	193.5607
Bull	13	7,970.3291	613.1022**
Error	351	40,755.0867	116.1113

**P < .01

abnormal spermatozoa. The peak in abnormal sperm production was reached in October and the low in February. Means for percent abnormal cells are plotted in Figure II. It appears that adverse effects of high temperature on sperm occur during the later phases of spermatogenesis or during early spermiogenesis. The results of damage seem to appear 4 to 6 weeks after exposure. Mukherjee and Singh (1966), in a study of the semen characteristics of Hariana bulls, found that the head length of sperm decreased and head width increased with an increase in environmental temperature. Significant monthly differences were found in percent abnormal cells by Mercier and Salisbury (1946) in a study involving Holstein and Guernsey bulls. Lasley (1943) and Lasley and Bogart (1943) found no increase in abnormal sperm in semen samples from Hereford bulls from May to September. Swanson and Herman (1944) and Phillips et al. (1945) found no significant effect due to month or season on percent of abnormal sperm.

The effect of month and bull on the percent of dead sperm was found to be highly significant ($P < .01$) in this study (Table VI). Orthogonal comparisons revealed highly significant differences ($P < .01$) in the percent dead sperm between August and all other months studied; fall and winter; and fall and winter and March. A graph of the monthly means for percent of dead sperm is given in Figure II.

TABLE VII

ANALYSIS OF VARIANCE FOR THE EFFECT OF MONTH ON BODY TEMPERATURE OF BRAHMAN BULLS

Source	df	SS	MS
Total	391	109.1188	
Weeks	27	32.8380	1.2162**
Months	7	7.4320	1.0617**
Weeks/months	20	25.4060	1.2703**
Bull	13	18.7370	1.4413**
Error	351	57.5438	0.1639

**P < .01

TABLE VIII

MEAN ATMOSPHERIC TEMPERATURES FOR AUGUST 1967-MARCH 1968

Month	Daily Maximum	Daily Minimum	Monthly
August	89.3	70.8	80.1
September	84.5	64.5	74.5
October	79.3	51.1	65.2
November	72.3	45.4	58.9
December	64.2	44.6	54.4
January	60.2	30.0	49.6
February	56.7	33.6	45.2
March	69.4	45.6	57.5

A reduction in dead cells may be noted as cool weather progresses into cold, indicating detrimental effects of elevated temperatures on sperm viability. These findings are not in agreement with Anderson (1945), Donaldson (1963), and Mukherjee and Singh (1966), who reported no monthly or seasonal effect on percent of abnormal sperm.

Body temperature was significantly affected ($P < .01$) by months, weeks within months, and bull (Table VII). Orthogonal comparisons for the effect of month revealed that body temperature was significantly greater ($P < .01$) in August than in the other months studied. A comparison with European breeds would be of value in helpint to determine if Bos indicus bulls are more efficient in regulating their body temperature through physiological differences.

Mean atmospheric temperatures for the months studied are given in Table VIII.

PART II

EFFECT OF SHORT-TERM HEAT STRESS ON SEMEN PRODUCTION AND QUALITY OF BRAHMAN AND ANGUS BULLS

Introduction

This experiment was conducted to determine the effect of short-term heat stress on Brahman bulls and to study the relation of heat stress between breeds. This study was conducted from November 1967 to January 1968 at the L.S.U. Climate Control Chamber, Gourrier Lane, and the L.S.U. Angus Barn, Nicholson Drive Extension, Baton Rouge, Louisiana.

Materials and Methods

Eight 2-year-old bulls were used. All bulls were maintained on group pasture and given a ration for proper growth and development.

The bulls were placed in a climate control chamber for 12 hours at a temperature of 104°F. and 35% humidity. During the 12-hour period in the chamber, all animals had access to water and hay.

A semen sample was obtained from each bull within

2 hours after removal from the chamber. After the initial semen sample was collected (week-1), the bulls were returned to the community pasture. Weekly semen samples were then collected for 7 weeks following exposure to the heat stress.

Semen samples were collected using the previously described electro-ejaculation technique, and the semen was evaluated as discussed earlier. Data were analyzed according to procedures described by Snedecor (1956).

Results and Discussion

Seven orthogonal comparisons of weekly treatment totals were made for each of the semen characteristics studied. Orthogonal comparisons included comparison of week-1 versus week-2; week-3 versus week-4; week-5 versus week-6; week-7 versus week-8; weeks 1 and 2 versus weeks 3, 4, 5, 6, 7, and 8; weeks 3 and 4 versus weeks 5, 6, 7, and 8, and weeks 5 and 6 versus weeks 7 and 8.

The effect of week and breed on semen volume was non-significant (Table IX). Significant differences ($P < .05$) were found for bull within breed. Comparisons of weekly totals were also non-significant.

Prior to this study, it was believed that no weekly differences would be found in semen volume because of the heat exposure treatment. Skinner and Louw (1966) found no significant difference in semen volume following exposure to 40°C. for 12 hours.

TABLE IX

ANALYSIS OF VARIANCE FOR THE EFFECT OF SHORT-TERM HEAT STRESS ON SEMEN VOLUME IN BRAHMAN AND ANGUS BULLS

Source	df	SS	MS
Total	63	66.4800	
Bull	7	17.6012	2.5144*
Breed	1	0.2500	0.2500
Bull/breed	6	17.3512	2.8918*
Week	7	7.7825	1.1117
Error	49	41.0963	0.8387

*P < .05

TABLE X

ANALYSIS OF VARIANCE FOR THE EFFECT OF SHORT-TERM HEAT STRESS ON TOTAL SPERMATOZOA PER EJACULATE IN BRAHMAN AND ANGUS BULLS

Source	df	SS	MS
Total	63	578,589.3511	
Bulls	7	84,950.1611	12,135.7373
Breed	1	15,835.0764	15,835.0764
Bulls/breed	6	69,115.0847	11,519.1807
Weeks	7	93,684.8498	13,383.5499
Error	49	399,954.3402	8,162.3334

Results of the present study indicated that differences in total sperm per ejaculate for all sources of variation were non-significant. Differences in sperm per mm^3 for week and breed were non-significant; however, the effects of bull and bull within breed were highly significant (Tables X and XI). Significant differences ($P < .05$) in total sperm per ejaculate and concentration were found in a comparison of weeks 1 and 2 versus weeks 3, 4, 5, 6, 7, and 8. Significantly greater ($P < .05$) numbers of sperm per mm^3 and per ejaculate were produced in weeks 1 and 2 than in the remaining weeks. This would seem to indicate that short-term heat stress results in fewer sperm being available for ejaculation. Spermatozoa in the vas deferens killed by heat stress may have been resorbed, thus resulting in fewer sperm being available for ejaculation. Results of studies by Casady et al. (1953) and DeAlba and Riera (1966) also showed a reduction in total sperm per ejaculate and are thus in agreement with the findings of the present study.

Differences due to bull within breed for the percentage of motile sperm were highly significant ($P < .01$). Differences in the percentage of motile sperm were non-significant for week and breed. The set of orthogonal comparisons for weekly totals for percent motility were also non-significant. The analysis of variance for factors

TABLE XI

ANALYSIS OF VARIANCE FOR THE EFFECT OF SHORT-TERM HEAT STRESS ON SPERMATOZOA PER MM^3 OF SEMEN IN BRAHMAN AND ANGUS BULLS

Source	df	SS	MS
Total	63	71,968.2344	
Bulls	7	21,207.1094	3,029.5870**
Breed	1	1,147.5156	1,147.5156
Bull/breed	6	20,059.5938	3,343.2656**
Week	7	12,928.6094	1,846.9442
Error	49	36,832.5156	751.6839

**P < .01

TABLE XII

ANALYSIS OF VARIANCE FOR THE EFFECT OF SHORT-TERM HEAT STRESS ON PERCENT MOTILITY OF SPERM FROM BRAHMAN AND ANGUS BULLS

Source	df	SS	MS
Total	63	14,774.6094	
Bull	7	5,683.9844	811.9977**
Breed	1	19.1406	19.1406
Bull/breed	6	5,664.8438	944.1406**
Week	7	1,915.2344	273.6049
Error	49	7,175.3906	146.4365

**P < .01

influencing percent motility is given in Table XII. A plot of weekly percentage motility means showed that while no significant breed differences existed (Figure III), sperm motility of the Angus bulls tended to be more adversely affected than was the sperm motility of the Brahman bulls. The greatest effect on Angus bulls was exhibited in week-4; for Brahmans, it was week-6. By the eighth week, motility values returned to levels approximately the same as those of the initial collection for both breeds.

Skinner and Louw (1966) in a similar study found that sperm motility declined sharply approximately 8 days after bulls were exposed to 40°C. for 12 hours, 24 hours, or 6 days. Motility in the 24-hour and 6-day groups continued to decline until the fifth and sixth week. All groups approached pre-treatment levels by the eighth week. Insulation of the scrotum of Hereford bulls for 72 hours by Austin et al. (1961) resulted in a decrease in sperm motility during the fourth to seventh week. Bulls exposed to high cyclic temperatures for 8 hours daily produced semen with reduced motility in a study by Johnston et al. (1963). Recovery from heat stress was more rapid in the Red Sindhi than in the Holstein and Brown Swiss breeds.

In the present study, weekly differences in the percent abnormal sperm were highly significant ($P < .01$), as were differences due to bull within breed. Breed effects

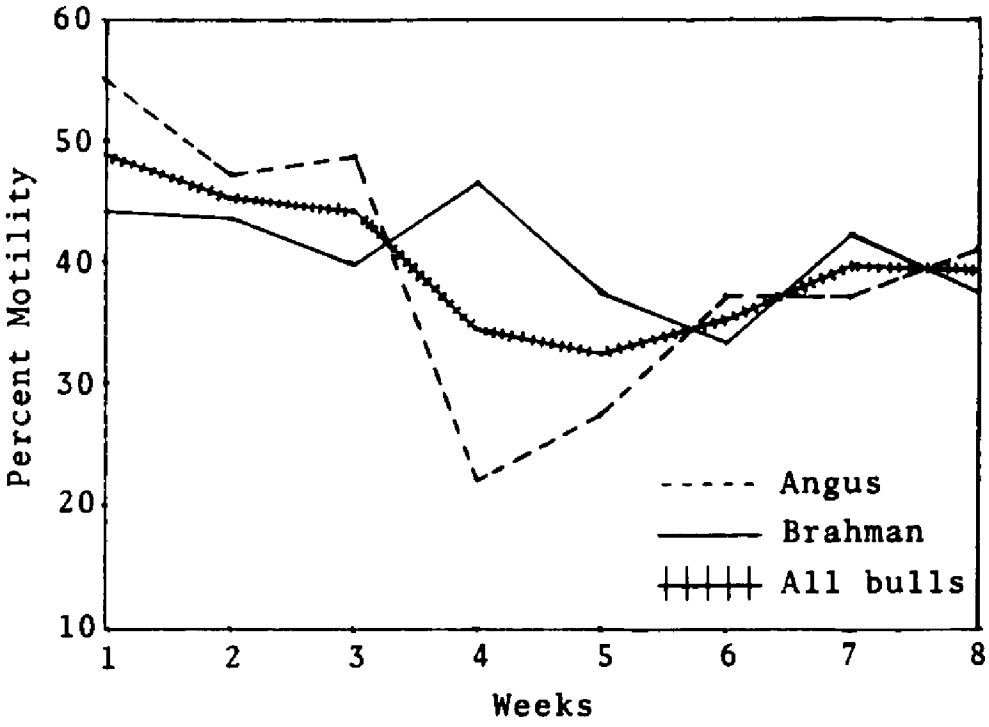

FIGURE III

Weekly Means of Percent Motility
for Bulls Subjected to Heat Stress

were non-significant. The analysis of variance for percent abnormals is given in Table XIII. Highly significant ($P < .01$) differences were found for a comparison of weeks 3 and 4 versus 5, 6, 7, and 8. Significant differences ($P < .05$) were found for a comparison of weeks 5 and 6 versus weeks 7 and 8. Figure IV is a plot of the weekly means for the percent abnormal spermatozoa. An increase in abnormals was noted for Angus bulls in week-2, with the increase reaching a peak in week-4. Thereafter, a rapid return to the initial collection level was noted. A similar pattern was found in Brahman bulls. However, an increase in abnormals was not observed until week-3. These findings seem to indicate that damage was limited chiefly to epididymal spermatozoa with some damage occurring during late spermatogenesis. Results of this study are in agreement with results of Langerlof (1934). An increase in abnormals was observed 10-40 days after scrotal insulation. Johnston et al. (1940) also noted an increase in abnormals beginning the second week after exposure to high temperatures. Skinner and Louw (1966) reported an increase in abnormals beginning the third week and lasting through the eighth week. A marked decrease in abnormals was noted during the seventh and eighth weeks but these were not sufficient to return the incidence of abnormals to its pre-treatment level.

TABLE XIII

ANALYSIS OF VARIANCE FOR THE EFFECT OF SHORT-TERM HEAT STRESS ON PERCENT ABNORMAL SPERMATOZOA FROM BRAHMAN AND ANGUS BULLS

Source	df	SS	MS
Total	63	15,576.1794	
Bulls	7	4,586.0056	655.1436**
Breed	1	21.6225	21.6225
Bulls/breed	6	4,564.3831	760.7305**
Week	7	6,148.5969	878.3709**
Error	49	4,841.5769	98.8076

**P < .01

TABLE XIV

ANALYSIS OF VARIANCE FOR THE EFFECT OF SHORT-TERM HEAT STRESS ON PERCENT DEAD SPERMATOZOA FROM BRAHMAN AND ANGUS BULLS

Source	df	SS	MS
Total	63	11,330.8086	
Bulls	7	1,336.1648	190.8806
Breed	1	32.9189	32.9189
Bulls/breed	6	1,303.2459	217.2076
Week	7	3,760.3073	537.1867**
Error	49	6,234.3365	127.2313

**P < .01

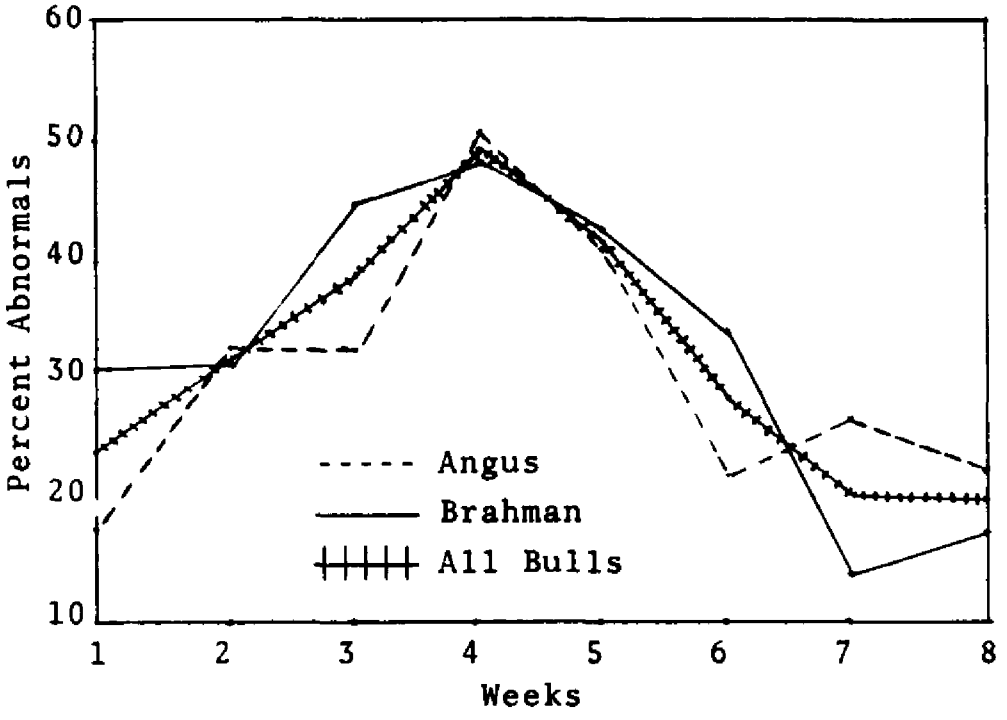

FIGURE IV

Weekly Means of Percent Abnormals for Bulls Subjected to Heat Stress

Highly significant weekly differences ($P < .01$) were noted in the percent dead spermatozoa (Table XIV). No differences were found for the effect of breed or bull within breed. Significantly greater ($P < .01$) numbers of dead sperm were ejaculated during the remaining weeks. A graph of the weekly means for percent dead is given in Figure V. A peak in the percent dead sperm in semen from Brahmans was reached during week-3. An increase in dead sperm in semen from the Angus bulls was observed at week-3, reaching a peak at week-4, and returning to the initial collection level by week-5. The increased incidence in the percent dead sperm in both the Angus and Brahman semen corresponded roughly to the decrease in motility discussed previously. Skinner and Louw (1966) reported a significant increase in dead sperm in bulls exposed to 40°C. for 12 hours. In their study, a maximum was reached from the third to the fifth weeks and recovery was completed by the eighth week.

No controls were available for use in this study; thus, no definite conclusions can be made regarding the effects of short-term heat stress on semen quality and production. However, a comparison of the results of this study with those of the monthly effect on semen quality (Part I) revealed that semen quality should be approaching its peak during the period that this phase of the study

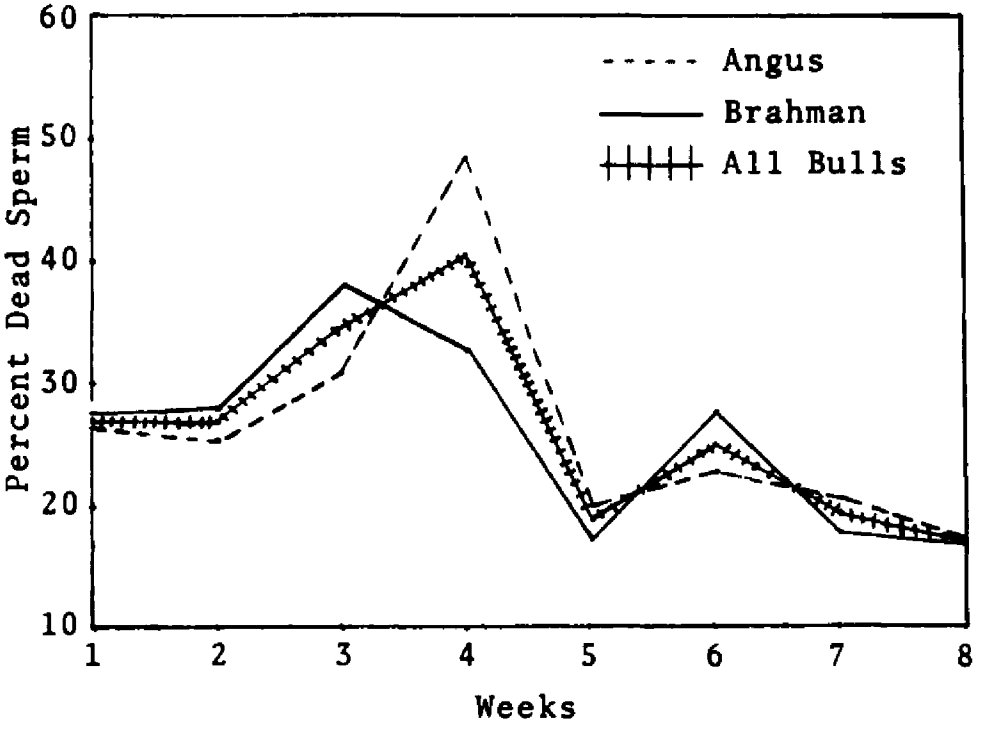

FIGURE V

Weekly Means of Percent Dead Sperm for Bulls Subjected to Heat Stress

was conducted. Thus, indications are that short-term heat stress was the reason for the effects observed in this study.

PART III

EFFECTS OF A PARTIAL EXHAUSTION TECHNIQUE ON SEMEN PRODUCTION

Introduction

This experiment was undertaken to obtain an estimate of sperm numbers available for ejaculation by Brahman bulls. In order to exhaust sperm reserves, high frequencies of collection are needed. Thus, the effects of high frequencies of collection on semen quality were studied. Knowledge of sperm reserves and the effects of frequency of ejaculation on semen quality coupled with information on seasonal changes in semen quality would be of great value in planning mating programs and in securing optimum use of bulls in artificial insemination studs.

Materials and Methods

Six 4-year-old Brahman bulls were used in this study. The bulls used were selected for their ease of collection from those animals which were employed in the ambient temperature study (Part I). The animals were managed under the same conditions as described previously.

Semen was collected twice daily from each bull on 3 consecutive days. A bull was ejaculated repeatedly until ejaculates were free of spermatozoa as indicated by clearness of the fluids. The bull was then allowed approximately an hour's rest and again ejaculated until sperm-free ejaculates were obtained.

The previously discussed semen collection technique was employed for collecting all semen samples. Semen samples thus obtained were evaluated according to those procedures already outlined. Analysis of data was made according to methods presented by Snedecor (1956).

Results and Discussion

Analysis of variance for the effect of a 3-day exhaustion technique on semen volume revealed a highly significant difference ($P < .01$) due to day (Table XV). Differences due to time were also significant ($P < .05$). The differences in volume due to bull and the day x time interaction were non-significant. Comparisons of the treatment totals for day-1 versus days 2 and 3 and day-2 versus day-3 revealed that a significantly greater ($P < .01$) volume of semen was produced on day-1 than on days 2 and 3. A high of 58.6 ml. and a low of 42.2 ml. of semen were produced on day-1 with a mean of 47.2 ml. for the six bulls collected. A mean of 21.5 ml. and 16.1 ml. of semen were produced on days 2 and 3, respectively. It was believed that these differences may have been due largely to the

TABLE XV

ANALYSIS OF VARIANCE FOR EFFECT OF A PARTIAL EXHAUSTION TECHNIQUE ON SEMEN VOLUME IN BRAHMAN BULLS

Source	df	SS	MS
Total	35	2,373.99	
Bulls	5	76.86	15.3720
Days	2	1,651.1850	825.5925**
Time	1	95.3877	95.3877*
D x T	2	79.7839	39.8919
Error	25	470.7734	18.8309

**P < .01 *P < .05

TABLE XVI

ANALYSIS OF VARIANCE FOR EFFECT OF A PARTIAL EXHAUSTION TECHNIQUE ON TOTAL SPERMATOZOA PER COLLECTION IN BRAHMAN BULLS

Source	df	SS	MS
Total	35	8,653,383.52	
Bulls	5	433,767.0233	84,753.4046
Days	2	5,275,718.6066	2,637,859.3033**
Time	1	896,430.2400	896,430.24
D x T	2	975,471.9267	487,735.9633**
Error	25	1,081,995.7234	43,279.8289

**P < .01

electro-ejaculation collection technique rather than to the frequency of collection. However, investigations by Boyd and Van Demark (1957), Singh and Prabhu (1963a, c), and Almquist and Cunningham (1967) found that semen volume decreased as collection frequencies increased. Baker et al. (1955) found that collections of one, two, or three times per week did not affect semen volume significantly.

In the present study, the effect of bulls did not significantly affect the total number of sperm per collection (Table XVI). A highly significant difference ($P < .01$) in the total number of spermatozoa per collection was found due to day, time, and the day x time interactions. A significantly greater ($P < .01$) number of sperm were ejaculated on day-1 than on day-2 and day-3. A high of 26.970 billion sperm and a low of 19.725 billion sperm were collected on day-1. On day-2 a high of 16.348 billion sperm and a low of 3.745 billion sperm were found. Analysis of data for day-3 revealed a high of 9.660 billion sperm and a low of 1.903 billion sperm. A mean of 22.472 billion sperm were ejaculated on day-1, a mean of 7.662 billion sperm were collected on day-2, and a mean of 5.103 billion sperm were collected on day-3. The 22.472 billion sperm per bull average found in this study as a result of partial exhaustion (day-1) was greater than the 18.1 billion sperm per bull reported by Boyd and Van Demark (1957) for

Holstein bulls and the 15.627 billion sperm per bull average reported by Walton and Edwards (1938). The average sperm per exhaustion found in this study was approximately one-half the 47.8 billion sperm per bull per trial found by Hale et al. (1953).

The findings of the present study would indicate that sperm reserves of a Brahman bull available for ejaculation is approximately 22 billion and that these reserves are replenished at the rate of 5-7 billion sperm per day. At mean levels found in the present study, four days would be required to restore the sperm reserves. Hale et al. (1953) found that within one week sperm reserves were restored to the pre-exhaustion level.

The analysis of variance for the effect of a partial exhaustion technique on semen concentration (Table XVII) revealed no significant differences in concentration attributable to day, time, bull, or the day x time interaction. Significantly greater ($P < .01$) concentration was observed on day-1 as compared with days 2 and 3. The differences in concentration between day-2 and day-3 were non-significant. These results indicate either a reduced number of spermatozoa available for ejaculation or a decrease in the sensitivity of the bulls to the ejaculatory stimulus.

Results of investigations by Singh and Prabhu (1963a, c) demonstrated significant differences in

TABLE XVII

ANALYSIS OF VARIANCE FOR EFFECT OF A PARTIAL EXHAUSTION TECHNIQUE ON SPERMATOZOA PER MM^3 OF SEMEN IN BRAHMAN BULLS

Source	df	SS	MS
Total	35	11,173.05	
Bulls	5	2,227.7	445.54
Days	2	1,380.27	690.1358
Time	1	590.49	590.4900
D x T	2	630.2733	315.1366
Error	25	6,344.3167	253.7726

TABLE XVIII

ANALYSIS OF VARIANCE FOR EFFECT OF A PARTIAL EXHAUSTION TECHNIQUE ON PERCENT MOTILITY OF SPERM FROM BRAHMAN BULLS

Source	df	SS	MS
Total	35	1,588.8889	
Bulls	5	272.222	54.4444
Days	2	251.3889	125.6944
Time	1	25.000	25.0000
D x T	2	12.50	6.25
Error	25	1,027.7778	41.1111

concentration as the frequency of ejaculation increased. However, Baker et al. (1955) and Almquist and Cunningham (1967) found that the differences in concentration were non-significant. Differences in the results of the present study and those of other investigators probably resulted from a difference in collection frequencies. Higher collection frequencies were used in the present study than in the earlier studies which found non-significant differences in concentration.

The effects of bulls, days, time and day x time interaction on percent motility were non-significant (Table XVIII). Orothogonal comparisons of the day effect indicate that semen collected on day-1 had a significantly greater ($P < .05$) number of motile sperm than did days 2 and 3. Day-2, however, did not differ significantly from day-3. Since high ejaculation frequencies were used in this study, it appears that these frequencies were responsible for the decrease in motility. A decrease in sperm motility due to frequency of collections was demonstrated by Singh and Prabhu (1963a, b); however, Baker et al. (1955) found that collection frequencies of up to three times per week had no effect on percent motile sperm.

Bulls, days, time and day x time interaction were found to be non-significant in their effect on percent of abnormal sperm (Table XIX). Orthogonal comparisons for

TABLE XIX

ANALYSIS OF VARIANCE FOR EFFECT OF A PARTIAL EXHAUSTION TECHNIQUE ON PERCENT ABNORMAL SPERMATOZOA IN BRAHMAN BULLS

Source	df	SS	MS
Total	35	1,314.9875	
Bulls	5	163.9691	32.7938
Days	2	91.5950	45.7975
Time	1	7.0225	7.0225
D x T	2	.9216	0.4608
Error	25	1,051.4793	42.0591

TABLE XX

ANALYSIS OF VARIANCE FOR EFFECT OF A PARTIAL EXHAUSTION TECHNIQUE ON PERCENT DEAD SPERMATOZOA IN BRAHMAN BULLS

Source	df	SS	MS
Total	35	134.8231	
Bulls	5	17.7981	3.5596
Days	2	1.8006	0.9003
Time	1	10.7803	10.7803
D x T	2	13.1514	6.5757
Error	25	91.2927	3.6517

days were also non-significant. Singh and Prabhu (1963c) found no differences in the percent abnormals when bulls were collected four times per week. No significant differences due to collection frequency were found by Baker et al. (1955) for bulls collected one, two, or three times per week. Langerlof (1936) did, however, find a decrease in sperm-bearing protoplasmic droplets as the frequency of semen collection increased.

Analysis of variance for factors affecting the percentage of dead sperm in semen is found in Table XX. Differences due to day, bull, time, and the day x time interaction were non-significant. Orthogonal comparisons for day-1 versus day-2 and day-3, and day-2 versus day-3 were also non-significant. Similar findings were reported by Baker et al. (1955), Singh and Prabhu (1963c), and Almquist and Cunningham (1967).

SUMMARY

This three-phase study was designed to investigate the effects of ambient temperature, short-term heat stress, and an exhaustion technique on semen production and semen quality of Brahman bulls.

Collections were made from fourteen 4-year-old Brahman bulls to study the effects of ambient temperature on semen quality. Analysis of the data from this study disclosed highly significant differences ($P < .01$) in semen volume for months, weeks within months, and bulls. Significantly greater ($P < .01$) volumes of semen were obtained in the fall than in the winter and in March than in the fall and winter.

The effects of bulls, weeks, and months on semen concentration and spermatozoa per ejaculate were highly significant ($P < .01$). Significantly greater numbers of sperm per ejaculate were produced in March than in the fall and winter months. Semen concentration was greater in the other months studied than in August and greater in March than in the fall and winter.

Differences in the percentage of motile sperm due to weeks, months, and bull were found to be highly significant ($P < .01$). The percent motility was significantly

lower ($P < .01$) in August than in the other months studied.

Highly significant differences for months, weeks within months, and bull were found in the percentage of abnormal spermatozoa. Orthogonal comparisons for August versus all other months, fall versus winter, and fall and winter versus March were significantly different ($P < .01$) from each other in the percent of abnormal spermatozoa.

The effect of week, month, and bull on the percent of dead sperm was found to be highly significant ($P < .01$). Highly significant differences ($P < .01$) were found in the percent dead sperm between August and all other months studied; fall and winter, and fall and winter and March.

Body temperature was significantly affected ($P < .01$) by months, weeks within months, and bull. Body temperature was significantly higher ($P < .01$) in August than in the other months.

No differences in semen quality were found between breeds when four Brahman bulls and four Angus bulls were exposed to 40°C. at 35% humidity for 12 hours. No weekly differences were found in volume of ejaculate or percent motility during the eight weekly collections following exposure. Differences due to bull and bull within breed were significant at the .05 and .01 levels, respectively. The percentage of dead spermatozoa was significantly higher ($P < .01$) during the third and fourth weeks following

exposure than during the remaining 4 weeks. The percentage of abnormal spermatozoa was significantly greater ($P < .05$) in week-4 than in week-3 and in week-5 than in week-6. Percentage of abnormal sperm was also significantly higher ($P < .01$) during weeks 3 and 4 than during the remaining 4 weeks, and also significantly greater ($P < .01$) in weeks 5 and 6 than during the seventh and eighth weeks following treatment.

Concentration was significantly greater ($P < .01$) in week-1 and week-2 following treatment than during the remaining 6 weeks. Total sperm per ejaculate was also significantly greater ($P < .05$) in week-1 and week-2 following treatment than during the remaining 6 weeks.

No controls were available to compare with the treated animals, thus making definite conclusions impossible regarding these findings. It appears, however, that the increase in percentage of abnormals and dead sperm for the bulls exposed to climatic stress was due to the short exposure to high temperature in the climatic-control chamber.

Results of the study involving a 3-day partial exhaustion technique on six Brahman bulls showed that volume, concentration, total spermatozoa, and percent motility were significantly higher on day-1 than on day-2 and day-3. No differences between the 3 days were found for percentage of

dead or abnormal spermatozoa.

These results seem to indicate that the quality of Brahman semen decreases under high ejaculation frequencies. With the electro-ejaculation techniques used, the spermatozoa reserves appeared virtually inexhaustible. Sperm production ranged from a high on day-1 of 26.970 billion cells to a low on day-3 of 1.90 billion cells.

Results of this three-phase study indicate that semen production and semen quality in Brahman bulls are adversely affected by heat stress. The greatest effects of heat stress appear to be manifest primarily 4 to 6 weeks after exposure.

SUGGESTIONS FOR FURTHER RESEARCH

On the basis of the information obtained in this study and from a review of the pertinent literature, the following suggestions for further research are given:

1. A comparison of Brahman semen quality collected via the electro-ejaculation and artificial vagina techniques would be of value.

2. Additional information on the gonadal and extra-gonadal sperm reserves of Brahman bulls is needed.

3. A study to determine if Brahman bulls and other beef breeds become refractory to the electrical stimulus provided by the electro-ejaculator would be of value.

4. An investigation of the effects of long-term semen collections with the electro-ejaculator technique on mating behavior of Brahman bulls would give additional information on the behavioral patterns of Brahmans.

5. It would be of value to determine if obesity during growth and development has any effect on semen production. This might aid in determining proper dietary regimens for developing young bulls.

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VITA

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EXAMINATION AND THESIS REPORT

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Title of Thesis: An Evaluation of Semen Production in Brahman Bulls

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

December 19, 1968